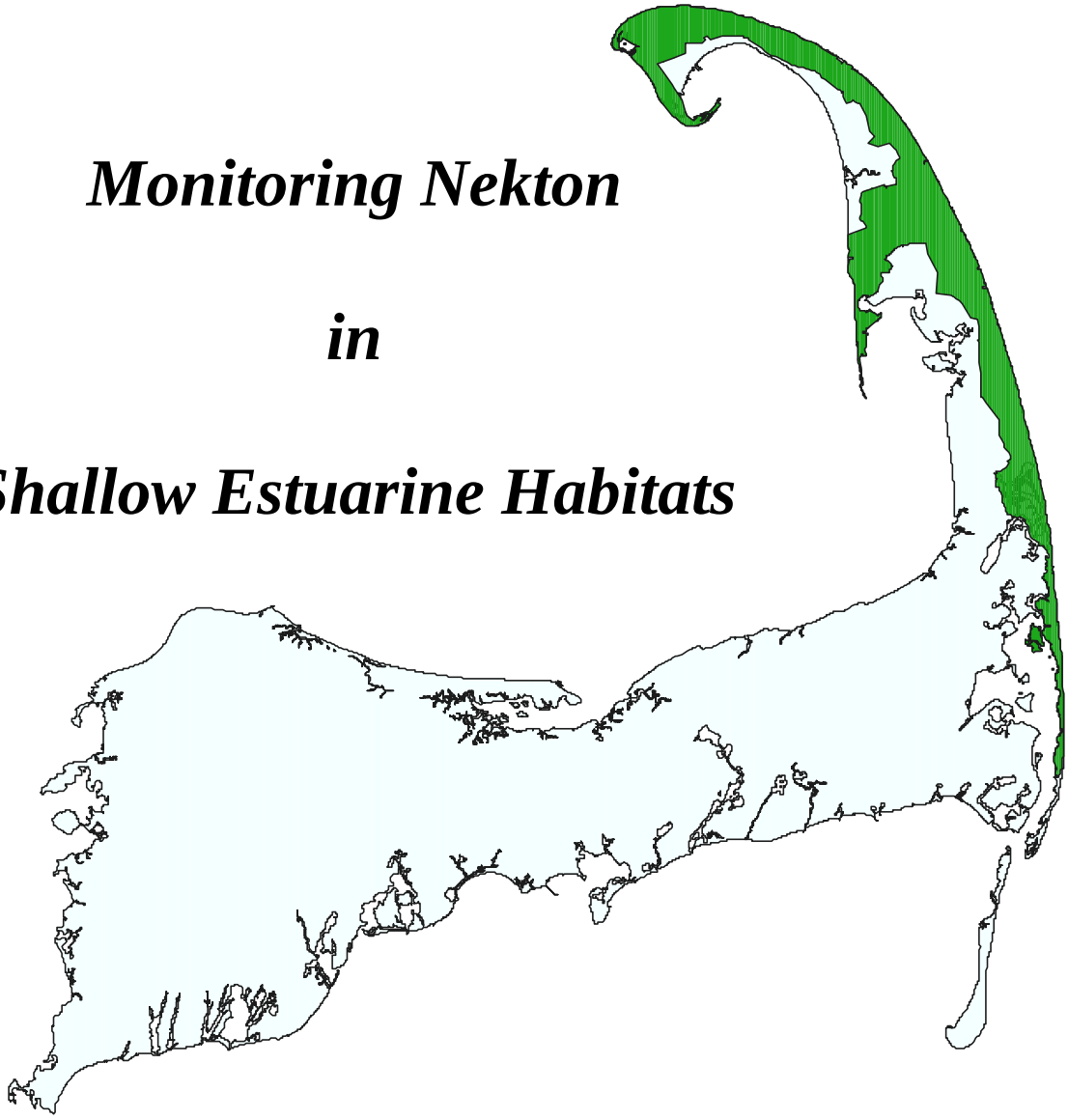
Monitoring Nekton

in

Shallow Estuarine Habitats



USGS Patuxent Wildlife Research Center

Cape Cod National Seashore

MONITORING NEKTON IN SHALLOW ESTUARINE HABITATS

A Protocol for the Long-term Coastal Ecosystem Monitoring Program
at Cape Cod National Seashore

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Long-term Coastal Ecosystem Monitoring Program
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PREFACE

Overview of Long-term Monitoring Program

Cape Cod National Seashore serves as a National Park Service prototype monitoring park for the Atlantic and Gulf Coast biogeographic region. The USGS, in cooperation with the National Park Service, is charged with designing and testing monitoring protocols for implementation at Cape Cod National Seashore. It is expected that many of the protocols will have direct application at other Seashore parks, as well as US Fish and Wildlife Service coastal refuges, within the biogeographic region.

The Long-term Coastal Ecosystem Monitoring Program at Cape Cod National Seashore is composed of numerous protocols that are relevant to the major ecosystem types (Estuaries and Salt Marshes, Barrier Islands/Spits/Dunes, Ponds and Freshwater Wetlands, Coastal Uplands). The nekton protocol is associated with the Estuaries and Salt Marshes component of the monitoring program. Other protocols being developed within the Estuaries and Salt Marshes component are related to nutrient enrichment, vegetation and habitat change, waterbirds, and sediment contaminants. The overall program is designed so that all of the protocols are interrelated. For example, information collected from the nutrient enrichment protocol or the vegetation change protocol may be especially relevant to interpreting observed trends in estuarine nekton. Roman and Barrett (1999) present a conceptual description of the entire monitoring program.

Protocol Organization

To maintain some consistency among the various monitoring protocols, each protocol is organized as follows. PART ONE of the protocol is intended to provide detail on the objectives of the monitoring protocol and to provide justification for the recommended sampling program. Incorporation of relevant literature and presentation of data collected during the protocol development phase of the project are used to justify a particular sampling design, sampling method, or data analysis technique.

PART TWO is a step-by-step description of the field, laboratory, data analysis, and data management aspects of the protocol. For example, PART TWO may simply state that samples are to be collected with a $1m^2$ enclosure trap from June through September. PART ONE provides a detailed justification as to why an enclosure trap was selected and why samples are being collected only in summer months, as opposed to seasonally.

Roman, C.T., and N.E. Barrett. 1999. Conceptual framework for the development of Long-term monitoring protocols at Cape Cod National Seashore. Technical Report, USGS Patuxent Wildlife Research Center, Coastal Research Field Station, Narragansett, RI. 59p. (http://www.nature.nps.gov/im/monitor/)

EXECUTIVE SUMMARY

Long-term monitoring of estuarine nekton has many practical and ecological benefits but efforts are hampered by a lack of standardized sampling procedures. This study develops a protocol for monitoring nekton in shallow (<1 m) estuarine habitats for use in the Long-term Coastal Monitoring Program at Cape Cod National Seashore. Sampling in seagrass and salt marsh habitats is emphasized due to the susceptibility of each habitat to anthropogenic stress and to the abundant and rich nekton assemblages that each habitat supports. Extensive sampling with quantitative enclosure traps that estimate nekton density is suggested. These gears have a high capture efficiency in most habitats and are small enough (typically 1 m^2) to permit sampling in specific microhabitats. Other aspects of nekton monitoring are discussed, including seasonal sampling considerations, sample allocation, station selection, sample size estimation, parameter selection, and associated environmental data sampling. Developing and initiating long-term nekton monitoring programs will help track natural and human-induced changes in estuarine nekton over time and advance our understanding of the interactions between nekton and the dynamic estuarine environment.

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Development of this protocol was supported by the USGS, with funds administered by the USGS Patuxent Wildlife Research Center at the University of Rhode Island. Special thanks are extended to the Superintendent and natural resource management staff at Cape Cod National Seashore for providing logistical support. Special thanks go the Evan Gwilliam for his dedicated assistance with data collection. James Heltshe (URI, Computer Science and Statistics) provided statistical advice. Mary-Jane James-Pirri (URI, Graduate School of Oceanography) and three anonymous reviewers are thanked for their detailed review of an earlier draft of this protocol.

PART ONE
Background and Justification for the Nekton Monitoring Protocol

INTRODUCTION

Threats to estuarine ecosystems include eutrophication, watershed development, wetland loss, overfishing, and other human-induced problems. Long-term monitoring of estuarine natural resources is needed to document the effects of anthropogenic impacts and to provide baseline datasets from unimpacted areas. In addition, long-term data are useful for differentiating natural and human induced variability and for formulating testable hypotheses regarding the ecology of estuarine species (Wolfe *et al.* 1987).

Nekton, defined here as an assemblage of fishes and decapod crustaceans, is an abundant estuarine fauna with unique responses to environmental change that make them desirable for inclusion in a coastal monitoring program. Development of the Index of Biotic Integrity (Karr 1981) and the Estuarine Index of Biotic Integrity (Deegan *et al.* 1997) attests to the value of monitoring nekton to document ecosystem level responses to anthropogenic stress. The foundation of these indices lies in the notion that fishes and decapods incorporate and reflect multiple ecosystem processes, and therefore indicate overall ecosystem integrity.

Nekton responds to ecosystem changes resulting from anthropogenic impacts. For example, fish abundance, species richness, and growth rates of the mummichog (*Fundulus heteroclitus*) increased in response to enhanced nitrogen loading (LaBrecque *et al.* 1996; Tober *et al.* 1996). Matheson *et al.* (1999) documented a shift in nekton community structure resulting from declines in seagrass distribution and standing crop in Florida. Several studies have also indicated that nekton responds rapidly (*e.g.*, within days to months) to the manipulation of salt marsh hydrology (Rey *et al.* 1990; Taylor *et al.* 1998; Able *et al.* 2000).

Estuarine nekton is an integral link among primary producers, consumers, and top predators and is likely to respond to either top-down or bottom-up estuarine perturbations. For example, nutrient enrichment (a bottom–up perturbation) could affect nekton by altering submersed vegetative habitats (Valiela *et al.* 1992; Harlin 1995). Conversely, removal of predatory fishes through overfishing (top-down) could induce responses in the forage or prey nekton guild (Carpenter and Kitchell 1985). Nekton also represents a significant portion of the diets of many piscivorous birds, economically valuable fishes, and, when in estuaries, marine mammals (Friedland *et al.* 1988; Sekiguchi 1995; Smith 1997).

There are many factors that make nekton a potentially useful and informative monitoring variable in estuaries. Figure 1 identifies some of the linkages between human-induced

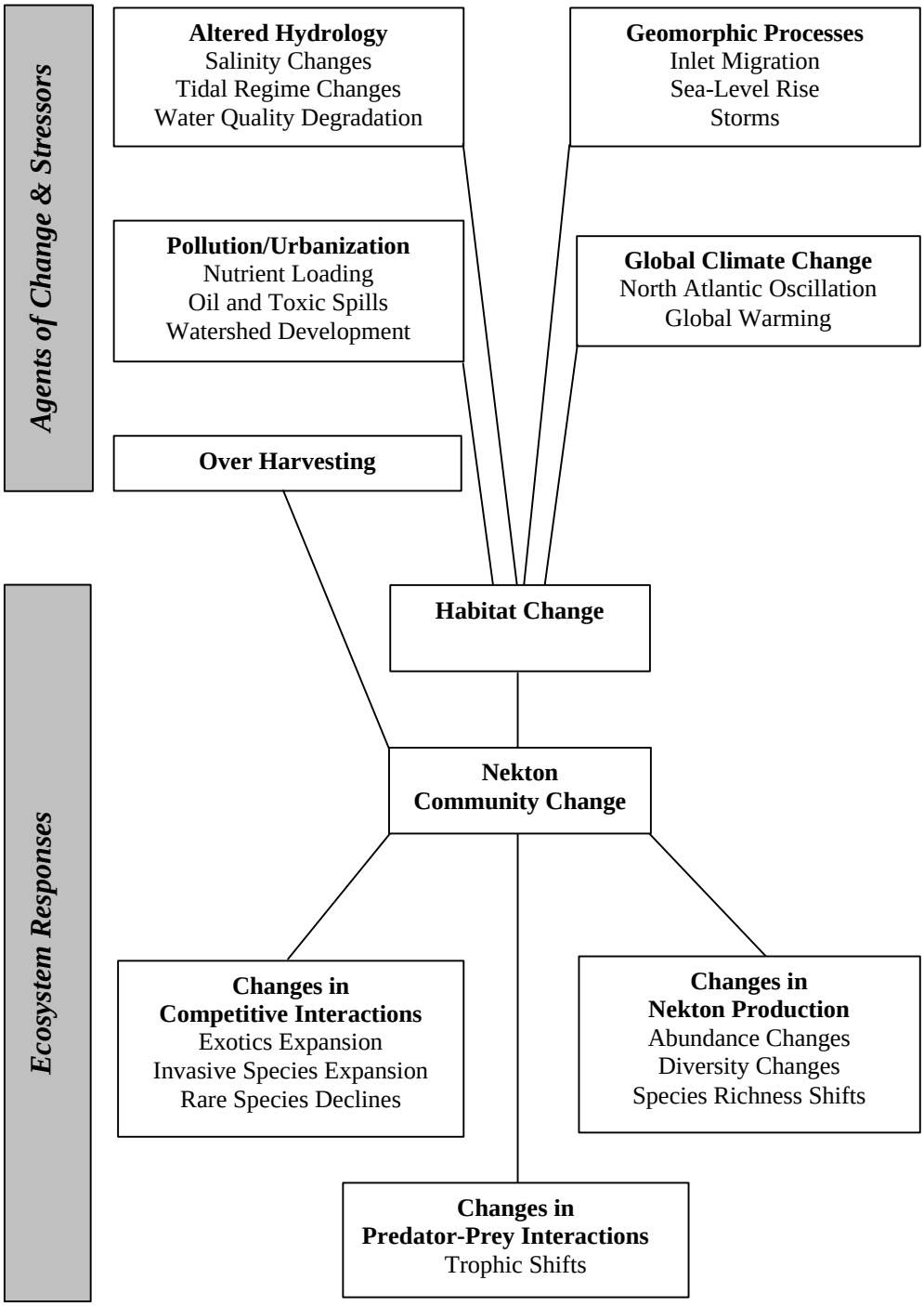

Figure 1. Linkages among environmental stressors and nekton responses in shallow estuarine environments.

and natural environmental stressors (*e.g.*, altered hydrology, nutrient enrichment, storms), associated changes in estuarine habitat structure, and responses of the nekton community. Given the coupling of nekton response to environmental stressors, the long-term monitoring program at Cape Cod National Seashore will include an estuarine nekton component.

The protocol presented in this report was developed for shallow subtidal habitats (<1m) that retain water throughout the tidal cycle. And more specifically, this protocol is intended for sampling shallow habitats within salt marshes (*e.g.*, creeks, pools) and shallow subtidal habitats associated with estuarine lagoons or bays, like seagrass beds, subtidal sand flats, and shallow algal beds. The methods proposed in this protocol are not appropriate for the sampling of nekton within estuarine intertidal flats, deep eelgrass beds, or gravel/rocky substrates. Development of this protocol was based on quantitative data that we collected from sampling programs in five southern New England estuaries (Figure 2, Table 1). Information gained from monitoring nekton should augment concurrent monitoring of other estuarine resources and processes. For example, monitoring only vegetation would not comprehensively describe the effects of salt marsh restoration, but monitoring vegetation along with nekton, birds, hydrology, and other variables would provide a more complete view of restoration responses and enable an evaluation of linkages among habitat characteristics and trophic levels.

MONITORING QUESTIONS

Long-term monitoring of nekton will be especially valuable for addressing questions related to habitat restoration and to long-term/large-scale ecosystem changes or processes.

Habitat Restoration

Recent studies document the rapid responses of nekton to restoration of tidal wetlands, both in New England and elsewhere (Rey *et al.* 1990; Vose and Bell 1994; Taylor *et al.* 1998; Raposa 2000). However, the complete effects of restoration on nekton are generally attained over several years, and therefore require long-term monitoring (Vose and Bell 1994; Raposa 2000). Long-term monitoring of nekton, as presented in this report, will help address the following questions as they pertain to restoration. These questions are specific to salt marshes at Cape Cod National Seashore, but they could apply to the restoration of other estuarine habitats, such as seagrass beds, and to other regions:

1. How do nekton communities in impacted salt marshes differ from reference marshes?
2. What are responses of nekton to restoration of impacted salt marshes?
3. What is the time frame for nekton communities in restoring salt marshes to achieve functional equivalency when compared to reference marshes?

4. How are changes in nekton related to changes in other ecosystem components such as vegetation, benthos, birds, and water quality during salt marsh restoration?
5. Can the response of nekton to restoration practices be predicted prior to implementation of restoration management?

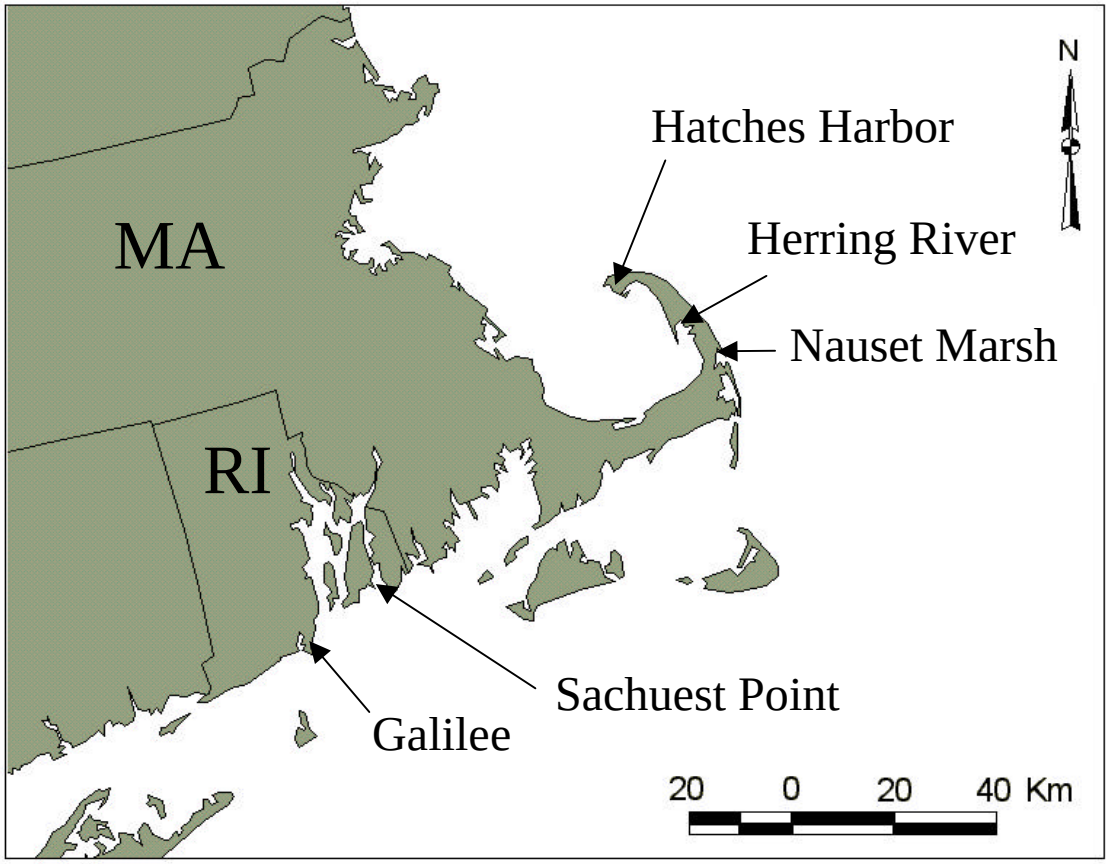

Figure 2. Location of the five study sites in southern New England where throw trap data were collected from 1997-1999.

Table 1. Locations and sampling regimes at five estuaries in southern New England. Sampling at all sites was conducted with throw traps only. Data are used from two distinct sampling programs at Galilee.

	Hatches Harbor	Herring River	Nauset Marsh	Sachuest Point	Galilee	Galilee
Location	Provincetown, MA	Wellfleet, MA	Eastham, MA	Middletown, RI	Narragansett, RI	Narragansett, RI
Geographic coordinates	42º06' N 70º23' W	41º 57' N 70º 04' W	41º 50' N 69º 57' W	41º28' N 71º14' W	41º22' N 71º30' W	41º22' N 71º30' W
Habitats sampled	Creeks, pools	Tidal channel	Marsh edge, eelgrass, creeks, pools	Creeks, pools	Creeks, pools	Creeks, pools
Sampling period	6/97-6/98	5/98-2/99	5/98-2/99	1997-1999 (Aug-Oct)	1997-1999 (Jun-Sep)	8/98-5/99
Sampling frequency	Biweekly	Seasonally	Seasonally	Monthly	Monthly	Seasonally
Total samples	770	240	500	300	392	160

Long-term/Large-scale Changes

Large-scale processes such as global and regional climate patterns and watershed-level development can impact nekton. On Cape Cod, the North Atlantic Oscillation, essentially a large-scale alteration in atmospheric masses between the subtropical high and the polar low, might impact nekton on a decadal scale through associated fluctuations in weather patterns or ocean temperatures (Hawk 1998). Ongoing development and nutrient enrichment can alter coastal habitats, often resulting in a shift from seagrass to algal-dominated habitats (Valiela *et al.* 1992; Kinney and Roman 1998), and thus affecting nekton. Long-term nekton monitoring will document the effects of these and other processes.

As salt marshes and other shallow estuarine habitats change in response to sea level rise, major storm events, and changing geomorphology and hydrology, nekton communities will be affected. Some salt marshes in the Chesapeake Bay are converting to open water habitat, reportedly related to sea level rise (Kearney *et al.* 1994; Ward *et al.* 1998). At Nauset Marsh in Cape Cod National Seashore (Roman *et al.* 1997) and along the Connecticut shore (Warren and Niering 1993), investigators have documented recent vegetation changes, perhaps in response to accelerated rates of sea level rise.

Long-term monitoring will also document the introduction or expansion of invasive species, interactions among invasive and native species, and changes in ranges of species. Finally, monitoring will enhance our understanding of the role that specific estuarine microhabitats play in supporting different life history stages of nekton.

Some specific monitoring questions that may pertain to evaluating the response of nekton to long-term or large-scale perturbations and processes are;

1. How do nekton respond to long-term human-induced or natural changes in the structure and distribution of estuarine habitats?
2. How do nekton respond to regional or large-scale processes such as global climate fluctuations, sea level rise, ocean temperature changes, or watershed development?
3. To what degree do nekton attributes vary inter-annually and how can natural variability be isolated from human-induced variability?
4. Are invasive species present in the nekton community, are new invasive species being introduced, are they changing in abundance, and are they affecting the structure and function of the estuarine nekton community?

SAMPLING METHODS

This section of the protocol provides justification and supporting documentation for various aspects of the protocol, including selection of habitats to monitor, sampling gear, sampling frequency (spatial, temporal, and sample size), and associated environmental monitoring parameters.

Habitat Selection

Seagrass beds and shallow water salt marsh habitats are especially important to include in a nekton monitoring program for several reasons. Seagrass beds provide nekton with abundant food resources and offer cover that increases protection from predation (Heck and Orth 1980a). Many studies report higher nekton abundances and/or higher species richness in seagrass beds compared to other estuarine habitats (Orth and Heck 1980; Weinstein and Brooks 1983; Heck *et al.* 1989; Connolly 1994; Raposa and Oviatt 2000). For example, in New York's Great South Bay 17 out of 40 species were more abundant in eelgrass compared to open sand areas (Briggs and O'Connor 1971). In Cape Cod's Nauset Marsh, eelgrass beds supported higher densities of nekton than unvegetated habitats adjacent to eelgrass beds or salt marsh habitats, such as tidal creeks or marsh pools, while species richness within eelgrass beds was comparable or greater than other common marsh-estuarine habitats (Figure 3).

Seagrass beds are highly susceptible to anthropogenic stress, especially nutrient enrichment. Increased nutrient loading into estuaries stimulates epiphytic and macroalgal

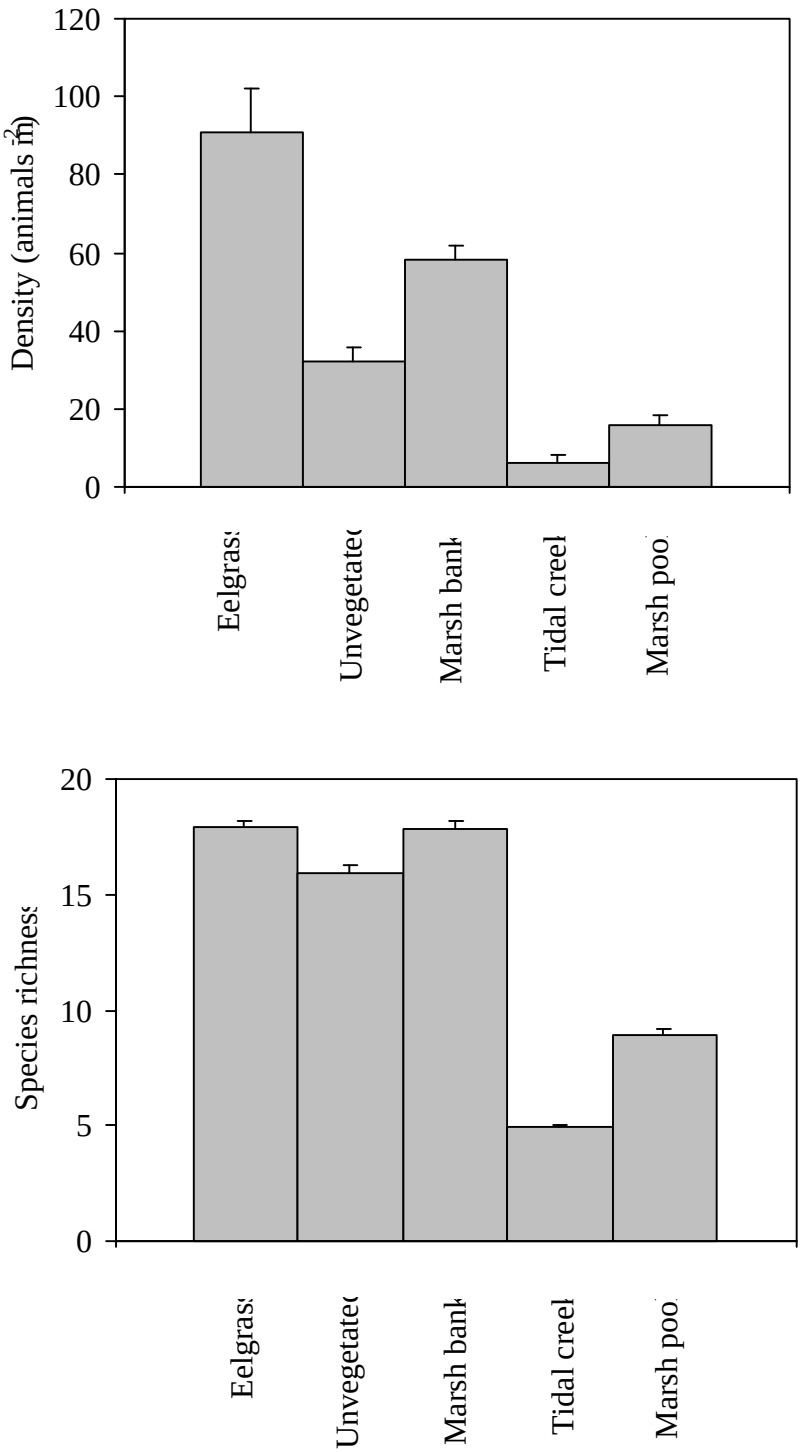

Figure 3. Nekton density (mean ± SE) and richness (± SE) in five shallow estuarine habitats. All data were collected with $1m^2$ throw traps at Nauset Marsh in October 1998. n=25 in each habitat, except marsh pools, n=50.

growth, often leading to shading of seagrass and eventual loss and die-off (Valiela *et al.* 1992; Dennison *et al.* 1993). There is evidence that moderate levels of macroalgae growth in seagrass beds is beneficial for some nekton (Gore et al 1981; Pihl Baden and Pihl 1984; Raposa and Oviatt 2000), and that macroalgae alone can provide surrogate habitat when seagrass is absent (Sogard and Able 1991). However, extremely dense macroalgal habitats, or conversely, unvegetated areas, generally do not provide habitat comparable to seagrass (Briggs and O'Connor 1971; Heck *et al.* 1989; Sogard and Able 1991; Connolly 1994; Raposa and Oviatt 2000). In response to watershed development and nutrient enrichment, there is compelling evidence that Cape Cod eelgrass beds are declining and being replaced by macroalgal habitat (Valiela *et al.* 1992; Short and Burdick 1996). Nekton clearly responds to changes in the structure of seagrass habitat over time, and thus, nekton sampling deserves inclusion in an estuarine monitoring program.

Salt marshes are also an important habitat for nekton, including juveniles of economically valuable species in some regions (Able *et al.* 1996; Minello 1999; Roman *et al.* 2000). Salt marshes provide food and refuge for estuarine species and there is evidence that they enhance the productivity of estuarine nekton assemblages (Boesch and Turner 1984). Within a salt marsh, nekton can potentially utilize a patchwork of habitat types including the marsh surface, tidal creeks, marsh pools, and the marsh edge. High nekton densities and utilization rates have been reported in all of these marsh sub-habitats (*e.g.*, Rountree and Able 1992; Smith and Able 1994; Able *et al.* 1996; McIvor and Rozas 1996; Minello 1999).

Salt marshes have also been heavily impacted by human activities, including extensive mosquito grid ditching (Bourn and Cottam 1950, Daiber 1986) and restriction of tidal flow by roads, causeways, and culverts (*e.g.*, Roman *et al.* 1984 and 1995, Rosza 1995, Burdick *et al.* 1997, Dionne *et al.* 1999). Today, extensive efforts are underway to restore natural tidal regimes to these degraded marshes by removing tide-restricting structures, excavating new habitats such as creeks and pools, and planting marsh grasses. Documenting the response of natural communities and marsh functions to restoration efforts requires the development of effective monitoring protocols.

Gear Selection

Many sampling gears are used to collect nekton in shallow (< 1 m) estuarine habitats. The large body of work devoted to gear comparisons and describing gear characteristics illustrates the importance of sampling gear selection (see review in Rozas and Minello 1997). The goals of individual projects will ultimately dictate gear selection, but pull nets (*e.g.* seines) and enclosure traps (*e.g.* throw traps) are two of the more common gears for sampling nekton in shallow water.

The capture efficiency of seines is generally low and is variable among different habitats (Rozas and Minello 1997). There is evidence that seines preferentially capture water column fishes and under-represent benthic nekton (Zedler 1990). In contrast, the capture

efficiency of throw traps is generally high and consistent among most habitat types (Rozas and Minello 1997). Throw traps may preferentially sample smaller nekton, while larger, faster, or less abundant species may be underrepresented in samples (Kushlan 1981). No gear can effectively sample the entire nekton assemblage in all habitats, but the high and consistent capture efficiency is a primary advantage of throw traps over seines. Higher capture efficiencies may also lower sample variance, and thus, sample size during monitoring (Peterson and Rabeni 1995).

Throw traps and seines sample a different area of habitat per unit effort. Most throw traps sample 1 m^2 (Figure 4). However, a small 10 m seine covers almost 80 m^2 in a single quarter-circle haul. Because they sample a larger area, seines might be expected to collect more species than traps. However, during this protocol's development we found that estimates of species richness using throw traps (13.9 species) and seines (16.9 species) in tidal creeks in a Cape Cod salt marsh were not different (Student's t-test; $p>0.05$; Raposa 2000). In estuaries south of New England, with different nekton species, studies comparing seine and throw trap methods should be done. It is known that New England salt marsh-dominated estuaries are dominated by resident species, while further south, seasonal transients and nursery species represent a greater portion of the nekton (Roman *et al.* 2000). Even so, if the goals of monitoring are to detect long-term changes in nekton and to document responses to human activities, collecting rare species may be less important than quantitatively collecting abundant resident species. By definition, residents spend their entire lives in the estuary or marsh and may be more reflective of ecosystem condition than transient species.

The large sampling area of seines can also be disadvantageous. In areas that are heterogeneous over small spatial scales (*e.g.*, meters), seines are not able to isolate and sample specific microhabitats. Instead, one sample may integrate collections from multiple microhabitats, such as seagrass and intermittent sand patches. Additionally, smaller creeks and pools can only be sampled by throw traps. For example, 45% of the creeks (by length) and 83% of marsh pools at Hatches Harbor marsh on Cape Cod were too narrow or small for proper seine sampling (Raposa 2000). Likewise, 86% of the pools in Cape Cod's Nauset Marsh that were sampled with the throw trap were too small to sample with a 10 m seine. Narrow creeks, small pools, and grid ditches are utilized by nekton and are important habitats that would go undocumented when sampling with only a seine. For these reasons, we concur with Rozas and Minello (1997) and suggest using throw traps for monitoring nekton in shallow (< 1 m) estuarine habitats. In deeper subtidal habitats, perhaps up to 1.5 m, a drop trap could be employed (Zimmerman *et al.* 1984), although the majority of seagrass and salt marsh habitats at Cape Cod National Seashore can be effectively sampled with a throw trap.

A 1 m^2 throw trap, as shown in Figure 4, is best used within sand or mud bottomed estuarine habitats. In gravel or rocky bottoms the seal between the trap bottom and the substrate is often not tight and capture efficiency decreases.

This protocol focuses on sampling nekton in subtidal habitats; however sampling on the intertidal marsh surface may also be desirable. A variety of gears are available for

Figure 4. TOP – 1m^2 throw trap used for quantitative sampling of nekton in shallow estuarine habitats.
BOTTOM – Bottomless lift net (6m^2) for sampling nekton utilizing the marsh surface. The nylon net is hidden within the marsh sediments during low tide. Then at high tide, when the marsh surface floods as shown here, the net is pulled up and nekton are captured. At low tide, when the water level recedes, nekton trapped within the enclosure are collected.

sampling in this habitat (*e.g.*, fyke nets; Dionne *et al.* 1999, Raposa 2000), bottomless lift nets (Rozas 1992), flume nets (McIvor and Odum 1986), and flume weirs (Kneib 1991). Bottomless lift nets have many characteristics in common with throw traps and we have found them effective for sampling nekton on the marsh surface. They are small (6 m^2), easy to use once installed, highly efficient, and relatively inexpensive to build (Figure 4). Their small size allows for sampling in specific marsh surface microhabitats (*e.g.*, *Spartina alterniflora* marsh edge, salt meadow) rather than collecting and integrating a sample from multiple habitat types, and allows a relatively large number of replicates compared to larger gears such as weirs and fyke nets.

Sampling Frequency

Spatial variability in nekton abundance is much higher than temporal variability in freshwater systems due to habitat heterogeneity (Peterson and Rabeni 1995). These authors found that collecting a larger number of samples on fewer dates would optimize sampling efforts, as opposed to taking a smaller number of samples spread out over multiple dates. To our knowledge, a similar detailed analysis of spatio-temporal variability does not exist for estuarine nekton. However, an analysis using nekton densities in tidal creeks from three southern New England salt marshes suggests that variability patterns may be similar for estuarine nekton (Table 2). Temporal variability in density among sampling dates was on average 21 times smaller than spatial variability (*i.e.*, variability among samples taken on the same sampling date). Because of this, we adopt the sampling strategy suggested by Peterson and Rabeni (1995) and suggest that a larger number of samples be collected on fewer dates to address spatial variability and improve sampling precision.

Spatial Frequency

There are at least two approaches for selecting nekton sampling stations in seagrass. One approach would be the collection of random samples solely from seagrass beds on each sampling date. The extent and distribution of seagrass beds changes temporally, so station locations must be flexible among sampling dates so that each 1 m^2 sample is located within seagrass. This method was used in studies in New Jersey (Sogard and Able 1991) and Florida Bay (Matheson *et al.* 1999). Another approach is to randomly establish permanent locations in an area that supports seagrass. With this method, sample locations may occur where seagrass is absent due to patchiness in cover. The first approach (non-permanent station locations always within seagrass) is appropriate if the goal of monitoring is to assess changes in seagrass-associated nekton assemblages. However, if the goal is to document overall changes in estuarine nekton over time in response to changes in seagrass habitat, including seagrass expansion, die-off, or replacement with macroalgae, then the second approach (permanent station locations) is more appropriate. We advocate the selection of permanent station locations for long-

Table 2. Spatial and temporal variability in nekton density in three New England salt marshes. All values are variance component estimates of temporal and spatial variability calculated using untransformed density data in the SAS variance component estimation procedure (PROC VARCOMP; SAS Institute, Inc., 1997). Nekton was collected in tidal creeks with throw traps between June and October 1997.

	Hatches Harbor		Galilee		Sachuest Point		Average	
	Spatial	Temporal	Spatial	Temporal	Spatial	Temporal	Spatial	Temporal
Total nekton	1977.0	95.0	1735.0	187.6	1048.1	47.1	1586.7	109.9
Total fish	1850.8	72.4	528.6	110.9	930.6	29.7	1103.3	71.0
Total decapods	252.6	0.0	1128.5	39.9	18.5	0.0	466.5	13.3
Fundulus heteroclitus	1814.7	66.8	170.3	34.2	890.4	0.0	958.5	33.7
Carcinus maenas	3.2	0.1	1.0	0.0	0.0	0.0	2.1	0.1
Fundulus majalis	1.5	0.1	17.2	1.9	0.0	0.0	9.3	1.0
Palaemonetes pugio	0.0	0.0	1123.2	42.0	18.6	0.0	570.9	21.0
Menidia menidia	0.8	0.0	48.9	3.2	2.2	0.9	17.3	1.4

term monitoring within Cape Cod National Seashore seagrass habitats. This approach can be flexible. For example, if seagrass distribution expands to new areas over time, additional permanent plots can be established, or if a seagrass area is covered by a barrier island overwash, then new permanent plots can be established elsewhere.

Selecting sampling stations in salt marshes is more involved because of multiple habitat types within the marsh ecosystem. Before monitoring is initiated a choice must be made between sampling in one habitat type that may be of special interest (*e.g.*, creeks) or in all habitats that are available to nekton (*e.g.,* creeks, pools, seagrass, marsh surface). Human perturbations may not affect nekton use of all salt marsh habitats equally; instead the impact may be most evident in a particular habitat. For example, differences in nekton density between the tide-restricted Hatches Harbor salt marsh and the adjacent unrestricted marsh were most pronounced in marsh pools, with higher densities noted in the tide-restricted marsh (Figure 5). Nekton utilization of creeks and marsh surface was similar on both the tide-restricted and unrestricted sides of the marsh. In this example, interesting differences in nekton utilization between the restricted and unrestricted marsh would have gone undocumented if only creeks or marsh surface were sampled. Unless there is a single marsh microhabitat that is of special interest, or if human impacts will clearly affect nekton in only one habitat type, samples should be collected from all available habitats when monitoring nekton in salt marshes. An appropriate design in this case would be a stratified random sampling approach, where habitat types are identified and sampling stations are located within each habitat type (Krebs 1989).

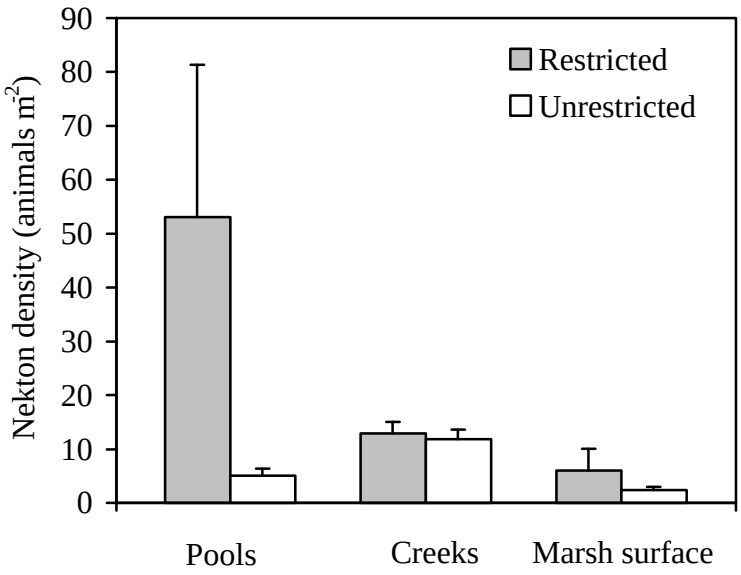

Figure 5. Nekton density (mean ± SE) from different habitats on the tide-restricted and unrestricted sides of Hatches Harbor salt marsh. Density estimates in creeks and pools were obtained with a 1 m^2 throw trap; estimates from the marsh surface were with fyke nets. Creeks and pools were sampled approximately twice a week for one year starting in June 1997, and the marsh surface was sampled twice a week from July through October 1997.

Sample Size

As previously noted, densities of estuarine nekton are highly variable, especially over spatial scales (Table 2). One way to address this variability and improve the ability to detect biological differences (*e.g.*, species richness, density) among treatments is to increase sample size. However, determining the appropriate sample size depends on a number of factors, such as the desired level of precision or if statistical comparisons are to be made, the desired difference among treatments one wishes to detect (Krebs 1989; Sokal and Rohlf 1981). Sample size also varies among different nekton species and depends on different attributes of the nekton community that are under consideration (*e.g.*, density, richness). A simple formula is available to estimate the required sample size to reach a desired level of precision (Snedecor and Cochran 1980):

$$N = (t^2 CV^2)/L^2$$

In this formula, N is the required number of samples, t is a constant that varies with the desired confidence level, CV is the coefficient of variation (CV = standard deviation/mean), and L is the desired level of precision.

We calculated the number of samples required to reach 20% precision around the mean (*e.g.*, SE ≤ 0.2 times the mean), at the 95% confidence level (t=1.96) for densities of total nekton and for common species in eelgrass, marsh edge, creek, and pool habitats (Figure 6). The 20% level has been used in other nekton sampling studies (Pihl Baden and Pihl 1984; Peterson and Rabeni 1995). Sample size clearly depends on the habitat and the level of community organization that is of interest (*e.g.*, common species vs. total nekton). When considering total nekton, the number of samples required in eelgrass beds and along the edge of fringing marsh or marsh-lined embayments was determined to be

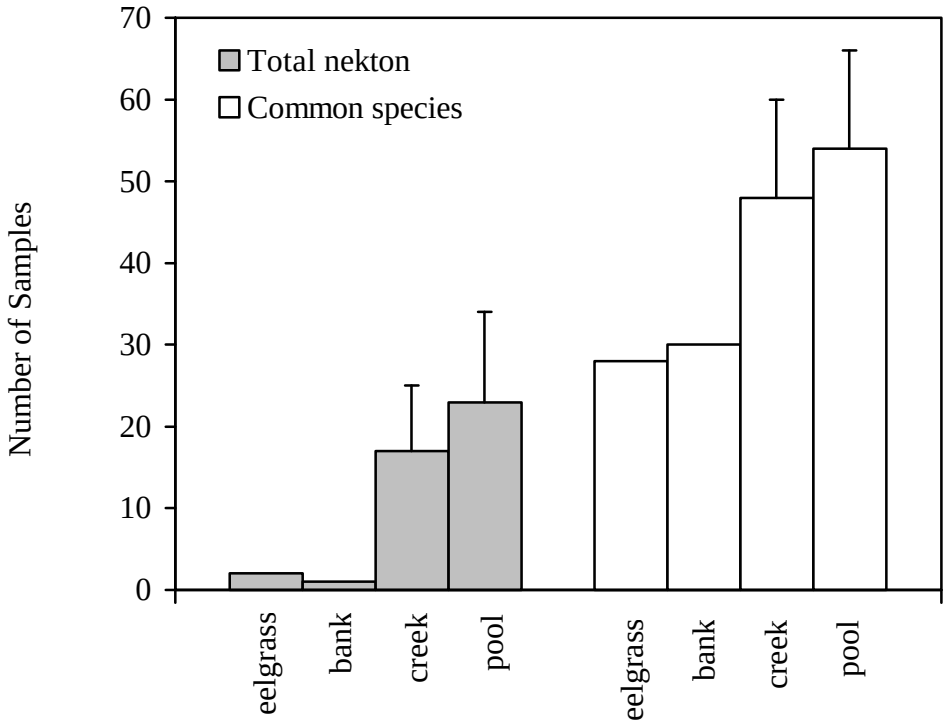

Figure 6. The number of samples needed to obtain a 20% level of precision at the 95% confidence level for nekton densities in four shallow estuarine habitats. Eelgrass and marsh edge sample size estimates were made using Nauset Marsh data. Creek and pool estimates were made from Nauset Marsh, Galilee, and Hatches Harbor and then averaged across sites (± SE). In each habitat, sample size estimates were made for total nekton density and for each common species (*i.e.*, species that were collected in ≥ 50% of the samples from that habitat) and then averaged across all species. Data were log (x+1) transformed prior to analysis.

relatively low. In contrast, sample size was substantially greater in tidal creek and pool habitats. If there is an interest in evaluating long-term trends in the density of individual species which are common (*e.g., Fundulus heteroclitus, Palaemonetes pugio*) than an even larger sample size would be necessary to attain 20% precision (Figure 6). Although not calculated here, it is expected that sample sizes for uncommon or rare species would be higher. As will be noted later in this protocol document, there is often a need to understand long-term trends in individual nekton species, and thus, based on the analysis presented in Figure 6, it is suggested that from 25 to 50 throw trap samples should be collected from each habitat of interest on each sampling date. Sample size would be toward the higher end of this range for habitats with high variability in nekton density (*e.g.,* marsh pools and creeks).

In addition to using a classic sample size formula to establish minimum sample size, we also conducted a power analysis. The objective of a power analysis is to determine the minimum number of sample replicates that are necessary to detect changes between nekton communities. Power is a function of the differences between two populations, sample size, alpha level of the test (probability of a type I error), and variability of the measured response. For this analysis, nekton community data (species compositions and abundance) from several southern New England marshes (Herring River, Hatches Harbor, Nauset Marsh all within Cape Cod National Seashore; Galilee salt marsh and Sachuest Point salt marsh, within Rhode Island), were collected using the throw trap from marsh creeks during the summer and fall. In this analysis the power of the permutation testing procedure outlined in Clarke and Green (1988) and Smith *et al.* (1990) was evaluated. This procedure allows statistical testing of equality between two nekton communities. The procedure uses a measure of similarity between two populations as a test statistic, and in this case a Euclidean distance similarity index (Krebs 1999) is used. Nekton communities similar in composition will have small distances and less similar communities larger distances between them. To look at power as a function of the similarity (as measured by Euclidean distance) between two populations, pairs of nekton data sets were selected that exhibited a range from similar (*e.g.*, Herring River in fall vs. summer) to quite different nekton composition (*e.g.*, Herring River restricted marsh in summer vs Galilee in summer). Using a pair of nekton communities we randomly selected samples of size 5, 10, and 15 from each nekton community and applied the permutation testing procedure to determine a reject or fail to reject decision for each trial. Two hundred (200) trials for each sample size for each pair of marshes were performed to determine the power to detect a difference between two marshes. Empirical power was estimated as the number of rejects by the permutation procedure out of the 200 trials.

From Figure 7 we can estimate the statistical power of detecting a difference between two nekton community data sets. As noted, with an n =5 there is a low power to detect differences, even for many cases where the differences between the two data sets are great. Increasing the sample size to n=10 or n=15 dramatically increases the power to differentiate two marsh nekton data sets, even between data sets that are quite similar. With a power above 0.9, there is a >90% chance of detecting a difference between data sets when a difference actually exists. With low power there is an increased probability of not detecting a difference when the data sets are actually different (*i.e.,* Type II error).

From the power analysis and associated power curve, an investigator could determine that if detecting subtle differences between nekton density data sets was of interest (*e.g.*, comparing nekton density in Marsh A over two consecutive sample years), then it may be appropriate to have a large number of replicates. If dramatic changes were to be detected (*e.g.*, comparing pristine Marsh A with highly impacted Marsh B), then perhaps a smaller number of replicates would be needed.

Determining a Type II error can be quite important in ecological studies, especially when evaluating environmental impacts on sites or when management actions are being considered. For example, consider a hypothesis that states that the nekton community of a particular marsh is the same in year 1 as in year 2, and based on a statistical test the null hypothesis (*i.e.*, there is no difference in nekton community between the marshes) is accepted). However, in actuality the nekton community in year 2 is different from year 1 (perhaps there was an increase in some invasive species), but by accepting the null hypothesis a Type II error was committed (accepting the null hypothesis when a difference truly exists). If the test were more powerful, the difference between the nekton

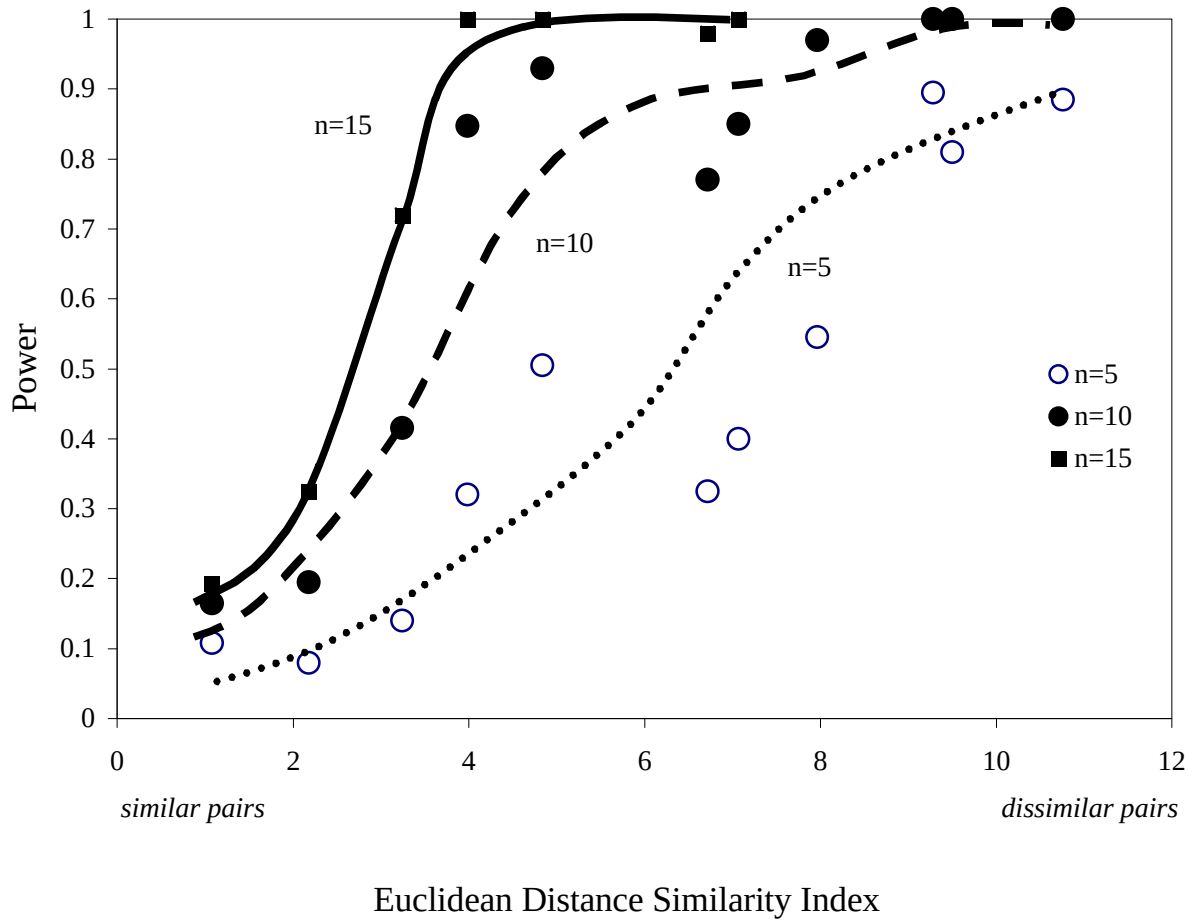

Figure 7. Power curves for sample sizes of 5, 10, and 15 with an alpha level of 0.05. Nekton density data from pairs of data sets that range in similarity from similar to dissimilar are compared.

communities would have been detected and some management action possibly initiated. Thus, in some instances it may be advisable to set a fairly high power, possibly 0.9 or above. This would result in a greater than 90% chance of detecting a difference between two data sets when differences actually exist.

If a Type I error is committed this means that the null hypothesis is rejected when in fact no difference exists. Therefore, falsely concluding that a difference in nekton communities exists between the marshes. In this case, possibly initiating management intervention for an invasive species, when in fact the nekton communities are indeed similar and the management action was not necessary. Type I errors are customarily set at either 0.05 or 0.10, indicating that there is a 5% or 10% chance, respectively, of falsely rejecting a null hypothesis.

To summarize, we have presented several estimates for determining an appropriate sample size. If based solely on the sample size formula and if interested in assessing changes in total nekton density, it is suggested that an appropriate number of throw trap samples may vary from n=5 for eelgrass beds to n=20 to 25 for marsh pools and creeks (Figure 6). However, if there is an interest in understanding trends in the density of individual or common nekton species, then the sample size would increase substantially, based on the sample size formula. The power analysis provides guidance on sample size if the intent is in detecting change in nekton community (*e.g.*, species composition and abundance), and the results are remarkably similar to the sample size estimates for density; suggesting an appropriate sample size of n=15. A power analysis was not performed on the nekton density or species richness data, but could be done. Based on these analyses and supported by our existing data sets that have successfully detected change in nekton density (and species richness) over temporal scales or comparing discrete marsh systems, it is concluded that investigators should seek an n=25 to 50, depending on the habitat type being monitored. If it is clear that only an analysis of change in total nekton is of interest, then sample size could be as low as n=5 to 15.

Temporal Frequency

Nekton density and richness were highest in either summer or fall in Hatches Harbor, Nauset Marsh, Herring River, and Galilee (Table 3). Similar peaks during warm temperatures are common in other temperate estuarine habitats (Pearcy and Richards 1962; Recksiek and McCleave 1973; Adams 1976; Cain and Dean 1976; Hoff and Ibara 1977; Heck and Orth 1980b; Orth and Heck 1980; Pihl and Rosenberg 1982; Pihl Baden and Pihl 1984; Ayvazian *et al.* 1992; Rountree and Able 1992; Able *et al.* 1996; Lazzari *et al.* 1999). In some cases the exact timing of nekton peaks depends on latitude and/or habitat type. For example, nekton abundance in eelgrass beds peaked in June in Chesapeake Bay (Heck and Orth 1980b, Orth and Heck 1980), but peaked in late summer and fall in Nauset Marsh (Heck *et al.* 1989). In Cape Cod and other southern New England salt marshes, abundance peaked in landward habitats (marsh pools, upstream tidal river) later in the year than in seaward habitats (marsh creeks, downstream tidal river) (Table 3), probably as a result of autumnal movements of some species into

Table 3. Seasonal patterns of nekton density and species richness in four New England estuaries. Spring = April to mid-June; Summer = mid-June to mid-September; Fall = mid-September to mid-December; Winter = mid-December to March.

		Density (animals m^{-2})				Species Richness			
Habitat	Site	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
Tidal creeks	Hatches	2.3	28.0	12.3	1.4	8	10	8	5
	Nauset	0.4	22.6	6.4	0.0	3	6	4	0
	Galilee	8.7	89.9	25.5	1.0	7	10	12	4
Marsh Pools	Hatches	14.6	13.0	70.3	13.5	7	7	6	5
	Nauset	3.7	16.3	16.3	0.5	8	9	8	3
	Galilee	3.7	225.7	49.9	8.4	6	9	9	4
Tidal River									
Downstream	Herring	40.0	78.5	28.4	3.2	10	13	7	3
Upstream	Herring	25.0	18.5	81.9	1.9	9	15	11	4
Eelgrass	Nauset	66.3	335.6	94.7	1.4	14	18	16	2

landward overwintering habitats (Fritz *et al.* 1975, Smith and Able 1994). A similar pattern was also observed in New Jersey salt marshes (Able *et al.* 1996).

Despite the variability in the timing of abundance and richness peaks, both parameters are generally highest between June and October in temperate estuaries. Therefore, monitoring efforts should be concentrated during this period to maximize information gained per sampling effort. However, there are species and processes unique to every season (*e.g.*, anadromous fish immigrations in spring) and timing of sampling should reflect the goals of individual monitoring programs.

To summarize, we suggest collecting samples during at least two periods: once during early summer (June-July) and once during late summer-early fall (August-October). The two sample times are supported by work in the Hudson River estuary where nekton assemblages collected in early summer were different from those collected in late summer (Able *et al.*, 1998). In Galilee and Hatches Harbor creeks, nekton communities in June or July differed from communities collected in August, September, or October at the same site 83% of the time (Analysis of Similarity, ANOSIM; $p<0.05$ in 15 out of 18 comparisons). Sampling in both early summer and late summer-early fall will more comprehensively document nekton use of the study area. In addition, since abundance and richness vary among sites and habitats this sampling scheme will improve the probability of sampling during peaks in nekton use.

Each sampling period should extend over multiple days (*e.g.*, 3-5 days). Nekton parameters can vary considerably over consecutive day periods in salt marshes (Varnell *et al.*, 1995). These authors showed that sampling on only one day would often produce inaccurate results, depending on the parameter in question. Sampling over multiple days will not only provide more accurate results, but also will allow for a larger sample size and increased sampling precision.

Some studies have demonstrated differences in estuarine nekton composition and abundance between day and night periods (Rountree and Able 1993, Heck *et al.* 1989). Using throw traps at Hatches Harbor, we documented significantly higher densities of green crabs (*Carcinus maenas*) at night (Table 4). However, densities of all other species were not different between day and night at Hatches Harbor, and we therefore recommend that samples only be collected during the day. This approach should provide accurate representations of the densities of most species in the study area, keeping in mind that some species, due to their diurnal rhythms (particularly decapods), may be underrepresented during the day. The logistics of daytime sampling are more accommodating for field personnel and day sampling facilitates comparisons with a larger number of datasets. However, night sampling can be initiated to augment regular daytime sampling if time and resources allow, or if a particular question can only be addressed by night sampling.

Table 4. Density of nekton in Hatches Harbor during the day and night. Data are from 18 stations sampled with a 1 m^2 throw trap during the day and then resampled at night in August 1997. *Carcinus maenas* densities were significantly higher at night (Student's t-test, $p<0.005$), but densities of all other species did not differ between day and night

	Density (animals m^{-2})	
Species	Day	Night
Fundulus heteroclitus	36.7	29.4
Carcinus maenas	0.3	1.5
Fundulus majalis	0.6	0.1
Menidia menidia	0.5	0.1
Crangon septemspinosa	0.3	0.1
Gasterosteus aculeatus	0.1	0.1
Anguilla rostrata	0.1	0.1
Mugil cephalus	0.1	0.0
Micropogonias undulatus	0.0	0.1
Alosa pseudoharengus	0.0	0.1
Palaemonetes pugio	0.0	0.1

Data Collected for Each Sample

The monitoring protocol we have outlined thus far is amenable to measuring nekton species composition, richness, density, and lengths. Species composition, richness, and density are obtained simply by identifying and enumerating all captured animals in each trap and conducting the appropriate statistical analyses (see Data Analysis section). By measuring nekton lengths, researchers can gain information on habitat use by different life history stages. For example, by measuring mummichog (*Fundulus heteroclitus*) sizes from Hatches Harbor throw trap samples, we demonstrated changes in the size distributions of this species throughout the year, emphasizing the influx of young-of-the-year in summer (Figure 8).

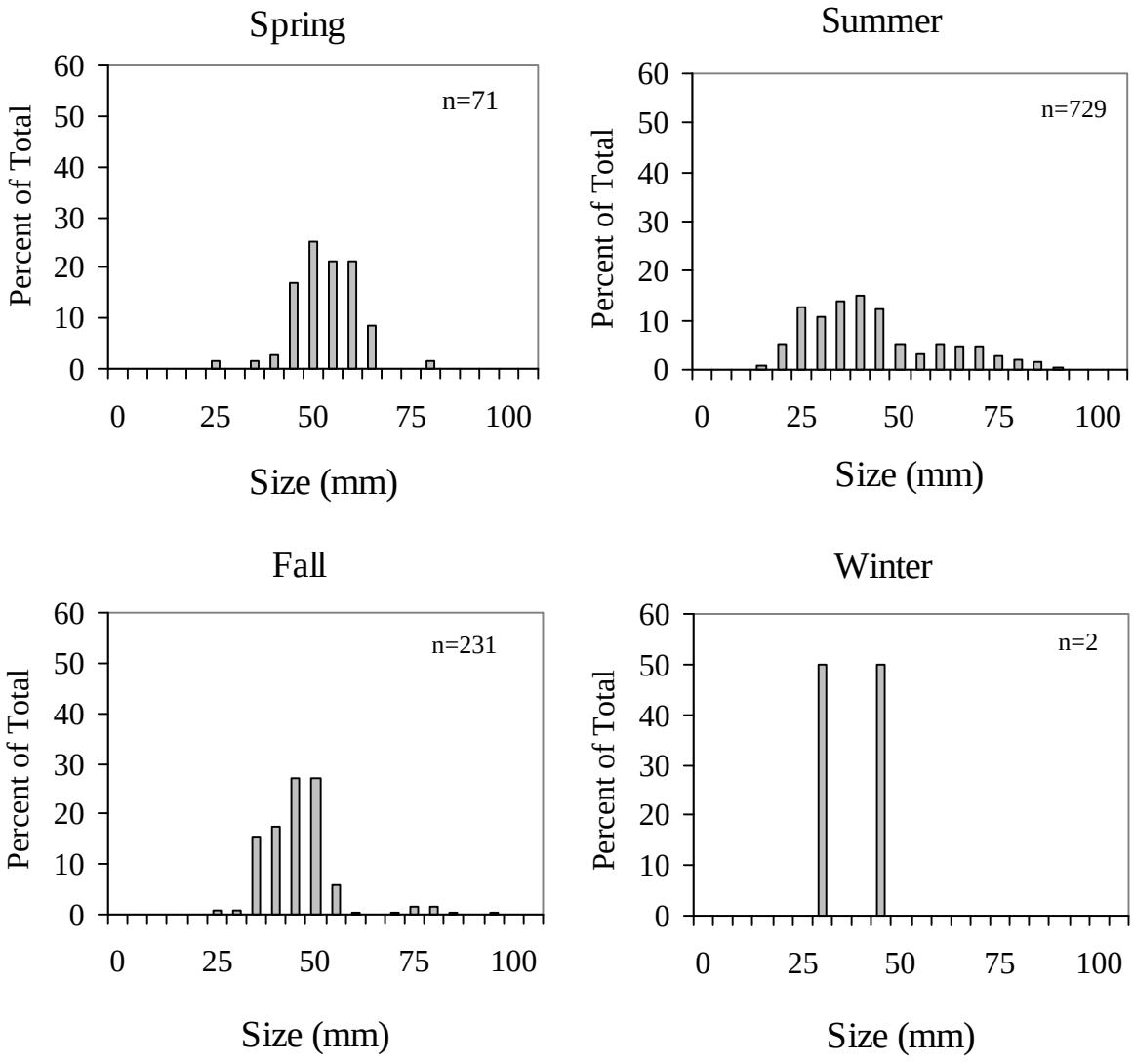

Figure 8. Length-frequency histograms for the mummichog (*Fundulus heteroclitus*) during four seasons in Hatches Harbor salt marsh. All fish were captured in tidal creeks with a $1m^2$ throw trap.

In all of our previous work, we typically measured the total length of a random sample of 30 individuals of each species per throw trap sample. These measurements add a considerable amount of processing time to each sample, especially during summer when nekton is abundant. The value of obtaining length measurements must be weighed against the time required to do so in each individual monitoring program. However, it appears that when a large number of throw trap samples are collected (*e.g.* >25), mean lengths obtained by measuring only 5 individuals per trap sample did not differ from mean lengths when 30 individuals are measured (Table 5). This was true for three different types of species: a decapod (*Palaemonetes pugio*), a ubiquitous-high density fish (*Fundulus heteroclitus*), and a patchy-high density fish (*Menidia menidia*). Although accurate length estimates can be obtained by measuring as few as 5 individuals per throw trap sample, we suggest a more conservative approach by randomly measuring at least 15 individuals of each species, particularly if distinct cohorts (*e.g.,* young-of-the-year and adults) are present or if analyses of trends in life history stages are desired.

Table 5. Mean lengths (mm) of common nekton species obtained by measuring between 5 and 30 individuals captured per throw trap sample at Galilee (N=26 throw trap samples). *Fundulus heteroclitus* were collected in July 1997, *Menidia menidia* were collected in August 1999, and *Palaemonetes pugio* were collected in August 1997. For each species, comparisons among treatments were made using one-way ANOVA, and the resultant *p* value is presented.

		Mean lengths (mm) of individuals per sample					
Species	*p*	n=5	n=10	n=15	n=20	n=25	n=30
Fundulus heteroclitus	0.99	30	30	30	30	30	30
Menidia menidia	0.66	41	42	44	45	45	45
Palaemonetes pugio	0.87	25	25	25	25	25	25

Associated Environmental Data

Measuring associated environmental variables will help define the sampling environment during monitoring. Certain variables may change with anthropogenic impacts over time; for example, lower dissolved oxygen levels with increased macroalgae from nutrient enrichment, increased salinity with tidal restoration, or conversely, decreased salinity with impoundment. By concurrently sampling basic measures, researchers can better define causal mechanisms for observed temporal changes in nekton.

Any number of environmental parameters can be sampled along with each throw trap sample. We suggest documenting vegetation cover or biomass, temperature, salinity, and water depth. Dissolved oxygen is also a common water quality variable that is often collected in conjunction with nekton sampling; however, single measurements are often difficult to interpret (a diurnal time series provides more useful information). When

monitoring nekton in seagrass, we also suggest developing a time series of geographic information system (GIS) habitat maps of the study site.

A visual estimate of vegetation cover within the trap is easily obtained using hierarchical cover classes (<1% cover, 1-5%, 5-25%, 25-50%, 50-75%, >75%) (Smartt *et al.* 1974, 1976; Kent and Coker 1992). Vegetation density can also be quantified by biomass estimates; collecting vegetation cores either within the trap before nekton is removed or immediately adjacent to the trap before any trampling occurs. Concurrent nekton and vegetation sampling is common in habitat utilization studies (Rozas and Odum 1987, Sogard and Able 1991, Matheson *et al.* 1999, Raposa and Oviatt 2000). Both the cover and biomass methods are rapid techniques. On a larger scale, habitat maps of the seagrass study area can be made using GIS. This would supplement *in situ* vegetation collections and further enable researchers to link nekton variability over time to habitat changes.

PART TWO
The Nekton Monitoring Protocol

SUMMARY

The estuarine nekton protocol (Table 6) recommends sampling exclusively with throw traps in shallow seagrass and salt marsh habitats (creeks, pools). There should be two daytime sampling efforts per year; one in early summer (June-July) and another in late summer-early fall (August-October), unless there are species or processes unique to other seasons that are of interest. The number of throw trap samples required depends on the habitat under examination, but generally between 25 and 50 samples should be collected from each sampled habitat during each sample period. Nekton composition, density, richness and length should be monitored in any program. Simple environmental parameters should be collected concurrent with nekton sampling, including temperature, salinity, water depth, dissolved oxygen, and vegetation cover.

This protocol is presented as a minimum for nekton monitoring. If additional time, personnel, or funds are available, supplementary sampling can be initiated; for example, additional sampling in spring, concurrent sampling on the marsh surface with a bottomless lift-net, or measurements of nekton biomass. There are also some limitations associated with the design. For example, sacrificing more sampling dates in favor of a large sample size during two sampling periods increases the possibility of missing short-term pulses of migrating species or newly hatching young-of-the-year. It would also not be possible to estimate growth rates by tracking modal lengths of cohorts over time. If growth rates (or production) were of interest, then a monitoring program with more sample dates would be appropriate.

One of the goals of presenting a model protocol is to inspire commonality among sampling programs in disparate geographic areas and to promote comparisons among datasets over space and time. However, this is a prototype protocol and is amenable to modifications to accommodate individual monitoring efforts. This protocol should serve to stimulate monitoring of nekton in shallow estuarine habitats to provide long-term, quantitative data sets to help evaluate the status of estuarine natural resources over time and in response to human-induced or natural habitat changes.

PROTOCOL

Site Selection and Sample Location

All sampling stations should be randomly selected prior to monitoring. This can be accomplished in a variety of ways, but two methods we use are described here. To select random sampling stations in subtidal marsh habitats, for example, we first plotted a GIS

Table 6. Protocol for monitoring nekton in two major shallow estuarine habitats – eelgrass beds and salt marshes. The protocol addresses spatial and temporal distributions of samples, sampling frequency, parameters of interest, and additional environmental data. T = temperature; S = salinity; D = water depth.

	Eelgrass	**Salt marsh**
Sampling gear	Throw trap	Throw trap
Season	Early summer; late summer	Early summer; late summer
Daily frequency	Over multiple days (2+ days)	Over multiple days (2+ days)
Annual frequency	1-3 yr intervals	1-3 yr intervals
Sampling design	Random or systematic permanent stations	Stations stratified by dominant habitats
Number of samples	>25	25-50+
Nekton parameters	Species composition, density, richness, length	Species composition, density, richness, length
Environmental data	GIS maps, vegetation cover, T, S, D	Vegetation cover, T, S, D

habitat map of the study site, overlaid with a grid. Each grid that landed on a tidal creek or other desired habitat was numbered sequentially. Random numbers between 1 and the total of numbered grid cells for each habitat of interest were then generated using a random number generator found in several spreadsheet programs. The random numbers correspond to the numbered grid cell, which in turn correspond to station locations on the map. Stations are then located in the field and marked with a 1 m oak stake and colored flagging and latitude/longitude coordinates recorded using a GPS. Station numbers should be indicated on the oak stake with a permanent marker (which will need to be remarked every season) or burned into the wood (branded).

The same method can also be used to select random stations in seagrass beds at certain intervals across the bed (*e.g.*, 10 m). Sample stations must be spaced far enough apart to insure independence. Stations can be marked using small floats attached by line to stakes in the sediment.

Sampling stations should be located and marked in the field and sampled during the early summer and late summer/early fall sampling intervals. Sampling station locations remain permanent for the duration of the monitoring program. However, as habitats change over time, such as expansion of a seagrass meadow onto a newly created flood tide delta, a new set of permanent stations can be established. Also, as seagrass areas are covered by

barrier island overwash processes, stations may need to be abandoned and others re-established.

Sampling Gear and Field Methods

Throw Trap Construction

The throw trap measures 1 m^2 x 0.5 m high (Figure 9). It consists of a frame made of 1 m long 2.5 cm horizontal aluminum bars attached with nuts, bolts, and lock-washers to 0.5 m long 2.5 cm angle aluminum bars. The four sides of the trap are surrounded by 3 mm mesh hardware cloth that is attached to the horizontal frame bars with thin gauge wire. If water depths are expected to exceed 0.5 m, the height of the trap can be extended to 1 m by attaching a skirt (3 mm mesh nylon netting) to the top of the trap. The skirt is equipped with float-cord along the top edge to ensure that the top of the skirt floats at the waters surface.

Nekton is removed from the trap using a 1 m wide x 0.5 m deep dip net that fits snugly within the throw trap. The net frame is constructed with 1.25 cm diameter aluminum rod. The rod is bent into the shape of the dip net with dimensions stated above and a 0.5 m piece of rod is left for the handle. The handle may be reinforced by slipping 2.5-5.0 cm diameter steel pipe over the aluminum rod handle. Netting (1 mm mesh) is attached to the dip net, either with numerous small cable ties, or by sewing with twine or wire. When reporting results from this method, investigators should cite a 3-mm mesh size, the mesh size of the throw trap. Use of a 1 mm mesh dip net facilitates collection of all nekton within the 1 m^2 frame.

Nekton Field Collection

Nekton sampling should occur at the same relative tide stage. All sampling in subtidal salt marsh habitats (*e.g.*, creeks and pools) should occur only after the marsh surface is drained of tidal water. If the marsh surface is flooded during sampling, densities of species that utilize the marsh surface will be underestimated in subtidal habitats. In marsh habitats, we generally begin sampling in seaward habitats where the marsh surface drains first, and then proceed to landward areas following the tidal prism. This method ensures that samples are collected at similar water depths throughout the marsh, and is thus one way to control for the effects of tide stage. Similarly, it is recommended that seagrass beds be sampled during periods when adjacent salt marsh areas are not flooded.

Figure 9. $1m^2$ throw trap. The investigator is sweeping the trap with the 1m x 0.5m dip net. Note the skirt of 3mm nylon mesh net attached to the top of the trap for sampling in deeper water.

Samples are collected by approaching to within 4-5 m of a marked station with the throw trap. The method used to approach stations will vary by habitat, but the primary objective is to not disturb or startle the nekton. For example in salt marshes, creek and pool stations are approached by crouching low and walking over the marsh surface, then waiting about 3 minutes before throwing the trap. The trap is thrown into the water by tossing it from the hip like a giant frisbee. The trap is then quickly pushed into the sediment to prevent escape of nekton from under the trap. In order to minimize disturbance, replicates are never taken from the same station in a single sampling period.

Once the sample is secured, nekton is removed by the large dip net. The net is slid downward into the trap, flush against the side of the trap nearest the researcher. The net is then moved across the trap with the forward edge of the net always remaining flush against the sediment until the opposite side of the trap is reached. In muddy sediments the dip net often goes through a thin layer of surface sediment, capturing buried nekton. The net is then moved upward out of the trap, again keeping the leading edge flush against the far wall of the trap. The dip net should be used from all four sides of the trap because nekton may be hiding in the trap corners. The dip-netting procedure is repeated until three consecutive dips do not capture any animals or if the first four dips come up empty. At this point the trap is considered empty.

Animals are processed as they are captured. All animals are identified to species in the field and immediately released at the same station. Individuals that are difficult to identify may be chilled on ice, preserved in 10% formalin, and returned to the laboratory for identification. In each sample, up to fifteen individuals of every species are measured to the nearest mm for total length (from the tip of the snout to the tip of the caudal fin for fishes; from the tip of the rostrum to the tip of the telson for shrimp) or carapace width for crabs (the distance between the two furthest points across the carapace). Nekton may be identified using any number of guides that are specific to the Atlantic coast and New England regions, including Bigelow and Schroeder (1953), Gosner (1978), and Robins *et al.* (1986).

Environmental Variable Field Collection

Once nekton is removed from a trap sample, environmental variables can be measured. Water temperature, to the nearest degree C, is measured using a stick thermometer or temperature probe. Likewise, salinity is measured, to the nearest part per thousand, using either a refractometer or water quality probe. Water depth in the trap is measured to the nearest cm using a meter stick. Alternatively, the sides of the trap can be marked off in centimeters and readings taken directly from the trap. The trap is often located on an uneven bottom, and thus, depth should be measured near each corner of the trap to obtain a mean depth value. Water depth is a simple measure and is useful for documenting changes in water depth over time. When monitoring restoration sites, where hydrology has been altered, this is a particularly important measure.

If macroalgae, marsh grass, or eelgrass are present within the trap, cover and species composition should be quantified. Prior to dip netting for nekton, the percent cover of

each plant species should be visually estimated according to the following cover class categories (<1% cover, 1-5%, 5-25%, 25-50%, 50-75%, >75%). These data provide a measure of the complexity of habitat available to the estuarine nekton.

Water clarity at Cape Cod National Seashore is always sufficient to use the visual cover estimate method; however, if sampling in regions with turbid waters and the vegetation can not be seen, then vegetation should be quantified by a biomass technique after Raposa and Oviatt (2000). Drift algae, if present, are obtained along with nekton during the dip netting. Algae are placed in plastic bags, returned to the laboratory, identified to species, and dried at 80^{o}C for dry weight determination (the data are expresses as dry weight m^{-2}). Submerged rooted vegetation is quantified by obtaining three cores (25 cm diameter) from immediately outside of the throw trap area. Vegetation collected is sieved in the field to remove sediment, placed in plastic bags, and returned to the laboratory for identification and dry weight determination.

Measures of water temperature, salinity, water depth, and plant cover are essential environmental data to collect in conjunction with each throw trap sample. Some investigators may elect to also collect other variables. Sediment composition (*e.g.* grain sizes and organic content) can be measured by extracting sediment cores and then processing according to Dean (1974). This information helps describe the habitat available to the nekton. Other variables such as creek width, creek order (*e.g.*, 1st order, 2nd order), pool size, adjacent shoreline type, distance of seagrass bed to shoreline, are easy measures and can enhance interpretation of the nekton data.

Data Management

Field data should be recorded in waterproof notebooks or on datasheets that are previously developed and printed on waterproof paper. Datasheets can be organized to the preference of individual researchers, but should include all information described previously in this protocol (*e.g.*, study site, date, station identification, habitat, species name, total number of individuals captured by species, lengths, comments, environmental parameters). An example of a sample datasheet is provided in Table 7. All field data should be transferred to digital format soon after sampling. Field data are easily incorporated into common spreadsheet programs that are designed for comprehensive data management.

After the data are entered it is important to carefully check the data for typos and mis-entries to insure the data are correct and to maintain quality assurance and quality control of the data.

Table 7. Sample nekton field data sheet.

THROW TRAP DATA SHEET

SITE:_____ DATE: _____ TIME:_____

STATION #:_____ SAMPLING CREW: _____

Water temp: _____ Salinity: _____ DO: _____

Water Depth: _____ Tide (circle one): Flood Ebb Vegetation (circle one): Yes No

Vegetation Species #1 _____ Veg. % Cover: <1% 1-5% 5-25% 50-75% >75%

Vegetation Species #2 _____ Veg. % Cover: <1% 1-5% 5-25% 50-75% >75%

NEKTON SPECIES & MEASUREMENTS

SPECIES #1 _____ Total # of individuals: _____

Talley (include measured fish): _____

LENGTHS: _____
(15)

SPECIES #2 _____ Total # of individuals: _____

Talley (include measured fish): _____

LENGTHS: _____
(15)

SPECIES #3 _____ Total # of individuals: _____

Talley (include measured fish): _____

LENGTHS: _____
(15)

SPECIES #4 _____ Total # of individuals: _____

Talley (include measured fish): _____

LENGTHS: _____
(15)

SPECIES #5 _____ Total # of individuals: _____

Talley (include measured fish): _____

LENGTHS: _____
(15)

Data Analysis Techniques

There are innumerable techniques for analyzing nekton data collected during monitoring. Here we describe some analyses that we have used with previous nekton datasets. Analyses of univariate measures (density, length) can be compared among treatments (*e.g.*, over time, among habitats, among sites) using analysis of variance (ANOVA) followed by post-hoc multiple comparison procedures to elucidate specific differences (*e.g.*, Least-squares Means test, SNK multiple range test). Density data are generally log (x+1) transformed to meet the assumptions of normality and equal variance that are associated with ANOVA. If the data fail to meet the parametric assumptions even after transformation, non-parametric tests can be used in lieu of ANOVA (*e.g.*, Kruskal-Wallis rank test, log-linear contingency tables).

For length data is should be noted that lengths are averaged per trap sample (trap is the replicate, not the individuals) and expresses as a mean $\pm$ standard error. Frequency distributions of the length data can be evaluated with the Kolmogorov-Smirnov goodness of fit test.

Multivariate measures such as community composition and species richness are analyzed with analysis of similarity (ANOSIM) and jackknifing techniques, respectively. ANOSIM is a non-parametric test, similar to multivariate analysis of variance (MANOVA) but without the generally unattainable assumptions (Clarke and Warwick 1994, Carr 1997). However, ANOSIM is only available in a few simple models (*e.g.*, one-way and two-way ANOSIM). A nice feature of ANOSIM is that significant differences among treatments can be followed with a similarity percentages (SIMPER) procedure that identifies species that are responsible for any observed differences. ANOSIM and SIMPER are included in the Primer statistical package (Carr 1997). Pairwise comparisons should be defined *a priori* and the alpha level adjusted (*i.e.*, Bonferroni adjustment) accordingly if necessary.

Species richness in a habitat or system is estimated using the procedure developed by Heltshe and Forrester (1983) and reviewed by Krebs (1989). Analysis between two treatments is compared by Student's t-test with Bonferroni adjusted alpha if pairwise comparisons are considered. This species richness jackknife procedure is more desirable than simply tallying the number of species collected since it takes into account sample size as well as the number of species collected. Alternatively, sample richness can also be measured (*e.g.*, the number of species in each sample, or the number of species per m^2).

When monitoring is conducted over multiple years, trend analysis techniques such as regression and correlation can be applied. When considering community composition, dissimilarity over time can be measured to quantify the amount of change in a given nekton community (Philippi *et al.* 1998).

Environmental data analysis techniques for the associated environmental data will depend on the monitoring questions. If the investigator is interested in how salinity, vegetation cover, or temperature affects nekton density or species richness, then simple correlations

can be performed. Multivariate procedures, such as canonical correspondence analysis can also be considered to explore relationships between species distributions and associated environmental variables (ter Braak 1986).

Equipment List

Equipment necessary to conduct the minimum nekton monitoring protocol is listed below. Additional gear will be necessary if other or different environmental parameters are to be included (*e.g.* sediment analyses).

Essential Gear	Additional Gear
Throw trap	Thermometer
Dip net	Refractometer/water quality probe
Tools (for repairs)	Corer (vegetation and sediment)
Identification guides	Labeled storage bags
Labeled specimen jars	Sieve
Formalin	GIS/GPS equipment
Cooler/ice	
Waterproof notebooks / datasheets	
Pencils	
Metric ruler / meter stick	
Hip boots/waders	

Personnel

With two persons, it will take approximately 1-3 sampling days to collect the 25-50 sample replicates that are required for each habitat, for each sampling interval. If only one salt mash ecosystem were included in the monitoring effort, such as Nauset Marsh, it would be expected to take a maximum of 9 days to sample eelgrass, marsh creek, and marsh pool habitats during a sampling period (3 days per habitat times 3 habitats). We suggest 2 sampling periods (early summer and late summer/early fall), and thus, a total of 18 field days would be required to complete the Nauset Marsh field sampling. Estimating the amount of time for other endeavors, such as data entry and report writing is difficult, and depends on the number of habitats sampled and personnel efficiency.

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